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No Heat Shock Protein Synthesis Is Required for Induced Thermostabilization of Translational Machinery

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For *Tetrahymena thermophila* cells to survive at 43°C, a normally lethal temperature, they require a pretreatment which either elicits the synthesis of heat shock proteins or one which brings about a change in the translational machinery of the cell such that is is not inactivated when transferred to 43°C. In this report I present evidence showing that the latter modification can occur in the complete absence of protein synthesis, indicating that heat shock protein production is not required for the induced thermostabilization of the translational machinery.

Thermotolerance has been defined (3, 8) as the induced capacity of cells to survive an otherwise lethal temperature after having been exposed to some stressful stimulus. Most often this pretreatment is a nonlethal, but heat shock protein- (hsp) inducing (16), hyperthermic shift. Other treatments which induce thermotolerance in a number of different organisms also induce the synthesis of hsps (4, 9, 10), which strongly implicates a causal relationship between hsps and thermotolerance.

It recently has been shown (7) that treatment of *Tetrahymena thermophila* cells with cycloheximide and emetine at concentrations which initially inhibit protein synthesis (and growth), but from which cells can recover (adapt [2, 15, 19]) and recommence growth while still in the presence of the drug, induces in these cells the ability to survive a direct shift from 30 to 43°C (>70% survival after 1 h). By contrast, naive (i.e., unstressed) cells are rapidly killed by the same shift from 30 to 43°C (<0.01% survivors after 1 h). This drug-induced tolerance to a 43°C exposure is as effective as a prior 60-min, 40°C treatment which is itself nonlethal but which induces hsp synthesis (7). The acquisition of the ability of drug-treated cells to survive at 43°C correlates with the recovery of the protein synthetic capacity of the cells. However, during the time that cells recover protein synthesis capacity, no hsp synthesis occurs (7). After this recovery the protein synthetic machinery of these cells, unlike that of unstressed cells, is no longer thermolabile at 43°C. Whereas a 30 to 43°C shift induces the rapid and quantitatively identical accumulation of hsp mRNAs in both unstressed and drug-adapted cells, only in the latter are they efficiently translated into hsps (7), which is presumably what allows the drug-adapted cells to survive.

From these and other observations I conclude that the acquisition of thermotolerance can have at least two aspects: (i) the prior accumulation of hsps, and (ii) the modification of the translational machinery such that it becomes resistant to normally heat-inactivating temperatures. For hyperthermic shifts well above the minimum lethal temperature (such as at 46°C, at which no protein synthesis ever occurs), only the first mechanism endows the cells with an enhanced survival capacity. For hyperthermic shifts to just above the minimum lethal temperature (42 or 43°C), either mechanism permits enhanced survival. That is, a cell can be preadapted to a normally lethal temperature because it already contains hsps or, alternatively, because it can rapidly and efficiently produce hsps upon reaching that temperature.

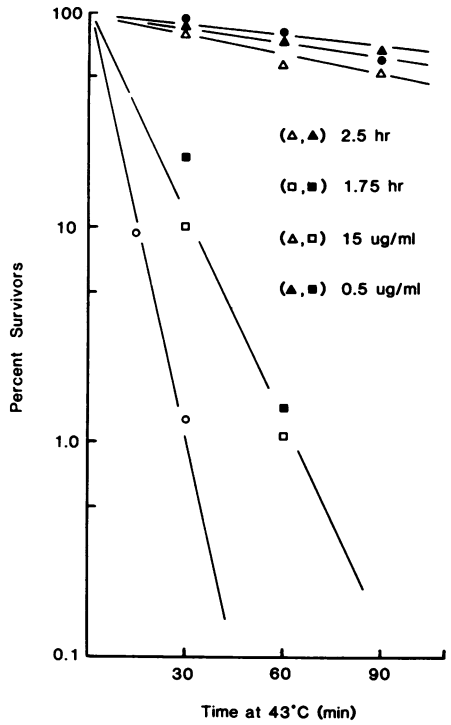

FIG. 1. Effects of two different concentrations of cycloheximide on the induction of tolerance to a 43°C heat shock. *T. thermophila* growing at 30°C were treated with cycloheximide at 0.5 μg/ml (▲, ■) or 15 μg/ml (△, □) (the details of growth conditions have been described previously [6, 7]). At the lower concentration of the drug, cells recovered protein synthetic activity within 2.5 h (7), while at the higher concentration, protein synthesis was completely abolished and was never restored (6, 7, 18). At 1.75 h (□, ■) and 2.5 h (△, ▲) after administration of the drug, cells were collected by centrifugation and washed twice in fresh growth medium, allowed to recover at 30°C for 5 min, and then transferred directly to 43°C. At various times after the transfer, fractions of cells were removed, and the cells were collected by centrifugation, suspended in 10 mM Tris, and allowed to recover at 30°C. Survivors (cells capable of further vegetative growth) were measured as described previously (6, 7). As positive and negative controls, cells growing at 30°C were shifted directly to 43°C (○) or were given a 1-h, 40°C treatment before they were shifted to 43°C (●).

TABLE 1. Effects of various drug treatments on the level of amino acid incorporation into proteins

Treatment (μg/ml) at T_0	Total cpm incorporated (%)/100,000 cells at the following times[a]		
	Label from 10 to 25 min after T_0	Label from 165 to 180 min after T_0	Wash out of drug at 180 min and label from 190 to 205 min after T_0
Control (none)	30,697 (100)	33,760 (100)	28,334 (100)
Cycloheximide (0.5)	1,850 (6)	36,221 (107)	26,159 (92)
Cycloheximide (15)	192 (0.6)	Less than background	23,377 (83)

[a] To cells growing at 30°C in 1% proteose peptone, cycloheximide was added to give a final concentration of either 0.5 or 15 μg/ml. At various times thereafter, fractions of cells were pulse-labeled for 15 min with [^{3}H]lysine, and triplicate samples were assayed for incorporation of the labeled amino acid into a hot trichloroacetic acid-precipitable form. Zero time (T_0) incorporation values (background values) were subtracted from the average values.

Because a nonlethal heat shock in *T. thermophila* (e.g., 40°C) not only elicits the synthesis of hsps but also endows the protein synthetic machinery with the ability to function at 43°C (7), I wished to know whether I could identify metabolic events which were common to heat shock and drug adaptation. I initially assumed that the thermoprotective modification of the translational machinery requires the synthesis of some new protein component(s) because the development of tolerance to 43°C following an exposure to cycloheximide or emetine paralleled the recovery of protein synthesis capacity. I now know that the converse is more likely true. Whatever modification(s) occur which allow translation in the presence of low levels of cycloheximide and emetine must simultaneously endow the protein synthetic machinery of the cell with the ability to function at 43°C. This fact is demonstrated by the data presented in Fig. 1. Cells were maintained in cycloheximide at 15 μg/ml at 30°C for 2.5 h, during which time essentially no protein synthesis was detectable (Table 1). When these cells were thoroughly washed from the drug-containing media, thereby allowing the recovery of protein synthesis, and then shifted to 43°C, they showed an induced thermotolerance. (In the previous study [7] the inhibitors were not completely removed; this explains the difference in the results reported here.) Quantitatively, this survival (defined as the ability to subsequently propagate vegetatively [6, 7]) is indistinguishable from that of cells starved for 2.5 in 0.5 μg of cycloheximide per ml, an inhibitory condition from which cells fully recover their protein synthetic activity while still in the presence of the drug (Table 1) (7). Cells treated with the two different concentrations of the drug for 1.75 h both showed partial, but identical, tolerance to a 43°C treatment. None of the treatments which endowed cells with any increased tolerance to 43°C provided the cells with an enhanced resistance to a subsequent shift to 46°C (see Fig. 2 for one example). Only a prior treatment which elicits the synthesis of hsps would do that (7) (Table 2 and Fig. 2).

Because under one set of conditions protein synthesis was not required for the stabilization of the translational machin-

TABLE 2. Effect of the presence or absence of cycloheximide during a nonlethal (40°C) heat shock on the induction of tolerance to a normally lethal heat shock.

Trial	Pretreatment[a]			Survivors		
	Cycloheximide	40°C	Cycloheximide present during 30°C recovery	43°C (1 h)	46°C (8 min)	hsps synthesized during pretreatment[b]
(a) 1.	−	−	−	<0.1	<0.1	−
2.	−	+	−	80 ± 7 (n = 3)	51	+
3.	+	−	−	<0.1	<0.1	−
4.	+	+	−	65 ± 15 (n = 3)	<0.1	−
(b) 1.	−	−	+	<0.1	<0.1	−
2.	−	+	+	72	44	+
3.	+	−	+	2[c]	<0.1	−
4.	+	+	+	60	<0.1	−

[a] Cells were taken through the experimental protocol diagrammed in Fig. 3. At the end of the 30°C recovery period cells were transferred to either 43°C for 1 h or 46°C for 8 min, and the percentage of surviving cells was determined.

[b] Synthesis of hsps was determined as described previously (7) by fluorographic analysis of electrophoretically separated proteins labeled during the pretreatment.

[c] These cells were presumably beginning to show the effects of drug adaptation, as they would have been in cycloheximide for about 1 h prior to the 43°C treatment.

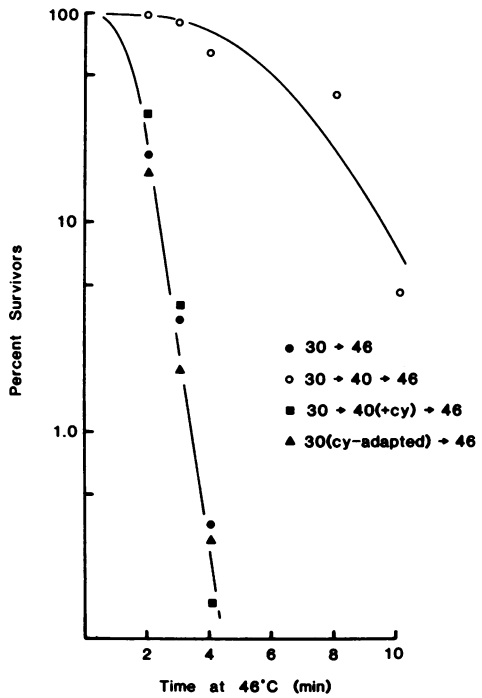

FIG. 2. Effects of various pretreatments on the survival capacity of cells exposed to a 46°C heat shock. *T. thermophila* cells growing at 30°C were (i) shifted directly to 46°C (●); (ii) shifted to 40°C for 30 min and then to 46°C (○); (iii) given cycloheximide at 15 μg/ml and shifted to 40°C for 30 min, and then washed free of the drug and shifted to 46°C (■); or (iv) given cycloheximide at 0.5 μg/ml for 150 min (a time sufficient for adaptation) and then shifted to 46°C (▲). In all cases the percent survivors was monitored at various times after the shift to 46°C.

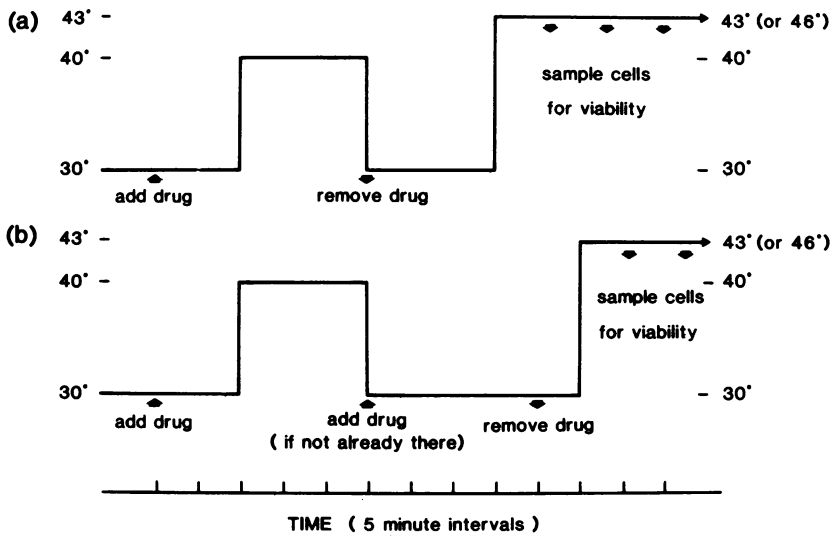

FIG. 3. Experimental protocol used to generate data reported in Table 2.

ery to a 43°C exposure, I asked whether the functionally similar alteration to the protein synthetic machinery which occurred in response to heat shock was also independent of protein synthesis. The following experimental protocol was followed to test that hypothesis (Fig. 3a). Cells in the early-logarithmic growth phase at 30°C were exposed to 15 μg of cycloheximide per ml for 10 min, a time which is more than sufficient to reduce amino acid incorporation to <1% that of the controls (Table 1) (5, 18). Cells were then shifted to 40°C for 15 min, a length of time which is sufficient to endow as much thermotolerance to a 43°C treatment as a 1-h, 40°C heat shock (compare positive controls in Fig. 1 and 4). The cells were collected by centrifugation, suspended in fresh growth medium, allowed to recover for 15 min at 30°C, and then shifted to 43°C for 1 h. Appropriate nonpreheated and nondrugged controls were run as well. Cells subsequently survived at 43°C whether or not cycloheximide was present during the 40°C pretreatment (Table 2). However, because the 40°C treatment induces the accumulation of hsp mRNAs (6, 7), it is possible that during the recovery period at 30°C in the absence of cycloheximide that hsps were synthesized, thus accounting for the results. Consequently, I repeated the previous experiment, but this time I allowed the cells to recover for 20 min at 30°C, with the cycloheximide still present, to allow for hsp mRNA degradation to occur (6), and then transferred them to fresh medium for 5 min and subsequently shifted them to 43°C (Fig. 3b). The results obtained with this protocol were essentially the same as before (Table 2). Because hsp mRNAs may be stable when the proteins for which they code are not present (1), results of the last experiment were not absolutely conclusive. Therefore, as a final check on the possibility that hsps are synthesized during the time in which cells are washed free of cycloheximide and before the shift to 43°C, I tested cells for their thermotolerance to a 46°C treatment (Table 2). Only the cells which had received a 40°C pretreatment in the absence of drug, and which therefore could accumulate hsps (6, 7), showed enhanced tolerance to the 46°C treatment (Fig. 2).

Cells which accumulated hsps could be shifted to 43°C and, in the presence of doses of inhibitors which abolish all capacity to continue synthesizing more hsps, survive at the >50% level for 1 h, after which time they are rapidly killed (7). The degree to which these cells are protected depends on the length of the prior 40°C heat treatment: the longer the pretreatment, the greater the initial protection (Fig. 4). This presumably is the result of the accumulation of more hsps when longer time is spent at 40°C. Therefore, as a further check on whether accumulation of any thermoprotective capacity (presumably hsp synthesis) occurs during the time cells are transferred from cycloxeximide-containing medium to fresh medium, the following experiment was performed. Cells were given a 10-min (to minimize the accumulation of either hsps or hsp mRNAs), 40°C pretreatment in the presence or absence of cycloheximide (15 μg/ml). They were then washed free of the cycloheximide and allowed to recover at 30°C for 20 min in fresh growth medium. One half of the cells were again treated with cycloheximide for 5 min and then all of the cells were shifted to 43°C and the survivability of the cells was monitored (Fig. 4). As before, cells receiving the 10-min, 40°C pretreatment in the presence or absence of cycloheximide survived identically when shifted to 43°C in the absence of cycloheximide (−/− and +/−). The cells which had received a 10-min, 40°C treatment in the absence of cycloheximide (−/+, 10′) before being transferred to 43°C in the presence of cycloheximide showed a small but distinct transient thermoprotection. It was decidedly less than those which had received a 60-min, 40°C treatment (−/+, 60′) and which had consequently synthesized a larger quantity of hsps. In contrast to these results, those cells pretreated at 40°C in the presence of cycloheximide, allowed to recover at 30°C in the absence of the drug, and then treated again with cycloheximide before being shifted to 43°C (+/+), were killed by the same kinetics as cells that never had a 40°C pretreatment. If these cells had accumulated hsp mRNAs during the 40°C treatment and then retained and translated them during the 20-min, 30°C recovery period, some tolerance to 43°C might have been expected. However, they displayed no evidence that they acquired any 43°C thermotolerance. All cells were also tested at 46°C, and the only ones that showed any enhanced tolerance to this temperature at all were those that had been treated at 40°C in the absence of cycloheximide (Fig. 2). I conclude from these data that a 40°C treatment as short as 10 min directly elicits the stabilization of the protein synthetic machinery of the cell, enabling it to function at 43°C, and that no new protein synthesis is required for this change to

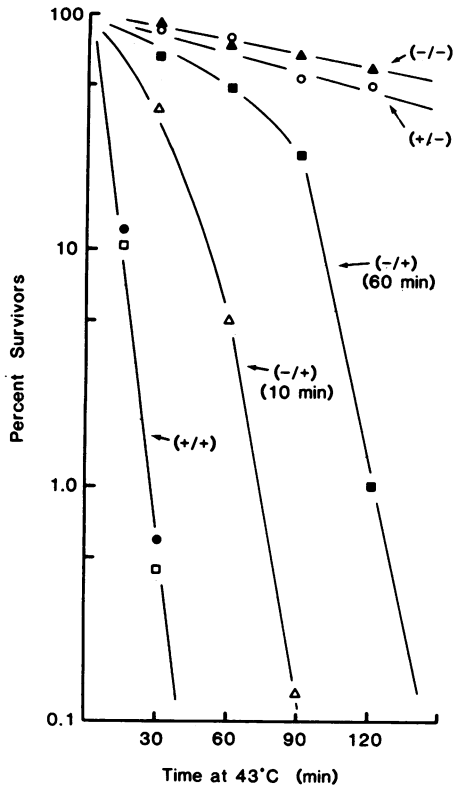

FIG. 4. Effects of the presence or absence of cycloheximide during both the 40°C heat shock and the 43°C heat shock on the survivability of cells at 43°C. Cells growing at 30°C were administered a 10-min, 40°C heat shock either in the presence (15 μg/ml, administered 10 min prior to the heat shock) or absence of cycloheximide. They were then collected by centrifugation, washed twice in fresh growth medium, and allowed to recover for 20 min at 30°C. The two cultures were split; to one half of each was added cycloheximide (15 μg/ml), and 5 min later all four cultures were transferred to 43°C. At intervals cells were removed from the flasks, and the percent survivors was determined. The first symbol in the parentheses in the figure indicate whether cycloheximide was present during the 40°C heat shock, and the second symbol indicates whether cycloheximide was present during the 43°C heat shock. In addition, cells which had received a 60- (rather than 10-) min, 40°C heat shock in the absence of cyclohexamide were allowed to recover at 30°C and then treated at 43°C in the presence of the drug (■). As a negative control, cells which received no prior 40°C pretreatment but which went through all the washing treatments were tested for survivability at 43°C (□).

occur. What this change might be, and if it is the same change elicited during drug adaptation, remains to be determined.

It has been shown previously that the repression of non-hsp mRNA translation which often occurs during the early stages of heat shock does not require hsp synthesis (13). What I have shown here is that the thermostabilization of the protein synthetic machinery which occurs during a nonlethal heat shock not only does not require hsp synthesis but requires no protein synthesis at all. A number of other systems such as those in soybean seedlings (11), *Drosophila melanogaster* cells in culture (12, 14), and CHO cells (17) display heat-inducible thermoprotection of their translational machinery. It will be interesting to know whether these cells use mechanisms similar to those employed by *T. thermophila* to accomplish this.

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LITERATURE CITED

1. **DiDomenico, B. J., G. E. Bugaisky, and S. Lindquist.** 1982. The heat shock response in self-regulated at both the transcriptional and posttranscriptional levels. Cell **31**:593–603.
2. **Frankel, J.** 1970. An analysis of the recovery of *Tetrahymena* from the effects of cycloheximide. J. Cell. Physiol. **76**:55–64.
3. **Gerner, E. W., and M. J. Schneider.** 1975. Induced thermal resistance in HeLa cells. Nature (London) **256**:500–502.
4. **Hahn, G. W., and G. C. Li.** 1982. Thermotolerance and heat shock proteins in mammalian cells. Radiat. Res. **92**:452–457.
5. **Hallberg, R. L., and E. M. Hallberg.** 1983. Characterization of a cycloheximide-resistant *Tetrahymena thermophila* mutant which also displays altered growth properties. Mol. Cell. Biol. **3**:503–510.
6. **Hallberg, R. L., K. W. Kraus, and R. C. Findly.** 1984. Starved *Tetrahymena thermophila* cells that are unable to mount an effective heat shock response selectively degrade their rRNA. Mol. Cell. Biol. **4**:2170–2179.
7. **Hallberg, R. L., K. W. Kraus, and E. M. Hallberg.** 1985. Induction of acquired thermotolerance in *Tetrahymena thermophila*: effects of protein synthesis inhibitors. Mol. Cell. Biol. **5**:2061–2069.
8. **Henle, K. J., and D. B. Leeper.** 1976. Interaction of hyperthermia and radiation in CHO cells: recovery kinetics. Radiat. Res. **66**:505–518.
9. **Li, G. C.** 1983. Induction of thermotolerance and enhanced heat shock protein synthesis in Chinese hamster fibroblasts by sodium arsenite and by ethanol. J. Cell. Physiol. **115**:116–122.
10. **Li, G. C., and Z. Werb.** 1982. Correlation between synthesis of heat shock proteins and development of thermotolerance in Chinese hamster fibroblasts. Proc. Natl. Acad. Sci. USA **79**: 3219–3222.
11. **Lin, C.-Y., J. K. Roberts, and J. L. Key.** 1984. Acquisition of thermtolerance in soybean seedlings: synthesis and accumulation of heat shock proteins and their cellular localization. Plant Physiol. **74**:152–160.
12. **Lindquist, S.** 1980. Varying patterns of protein synthesis in *Drosophila* during heat shock: implications for regulation. Dev. Biol. **77**:463–479.
13. **Lindquist, S.** 1981. Regulation of protein synthesis during heat shock. Nature (London) **293**:311–314.
14. **Peterson, N. S., and H. K. Mitchell.** 1981. Recovery of protein synthesis after heat shock: prior heat treatment affects the ability of cells to translate mRNA. Proc. Natl. Acad. Sci. USA **78**:1708–1711.
15. **Roberts, C. T., and E. Orias.** 1974. On the mechanism of adaptation to protein synthesis inhibitors by *Tetrahymena*. J. Cell Biol. **62**:707–716.
16. **Schlesinger, M. J., M. Ashburner, and A. Tissieres (ed.).** 1982. Heat shock from bacteria to man. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Sciandra, J. J., and J. R. Subjeck.** 1984. Heat shock proteins and protection of proliferation and translation in mammalian cells. Cancer Res. **44**:5188–5194.
18. **Sutton, C. A., M. Ares, and R. L. Hallberg.** 1978. Cycloheximide resistance can be mediated through either ribosomal subunit. Proc. Natl. Acad. Sci. USA **75**:3158–3162.
19. **Wang, T.-C., and A. B. Hooper.** 1978. Adaptation to cycloheximide of macro-molecular synthesis in *Tetrahymena*. J. Cell. Physiol. **95**:1–12.